

Claims

1. A method to produce an immunoglobulin with fully human variable region or an analog thereof, specific for a desired antigen, which method comprises:
 - 5 administering said antigen or an immunogenic portion thereof to a nonhuman animal under conditions to stimulate an immune response, whereby said animal produces B cells that secrete immunoglobulin specific for said antigen; wherein said nonhuman animal is characterized by
10 being substantially incapable of producing endogenous heavy or light immunoglobulin chain variable regions, but capable of producing human immunoglobulin variable regions; and
 - recovering said immunoglobulin or analog.
- 15 2. The method of claim 1 wherein said recovering step comprises recovering polyclonal immunoglobulin from said animal.
3. The method of claim 1 wherein said recovering step comprises immortalizing B cells from said
20 animal, screening the resulting immortalized cells for the secretion of said immunoglobulin, and
 - 1) recovering immunoglobulin secreted by said immortalized B cells, or
 - 2) recovering the genes encoding at least the
25 variable region of said immunoglobulin from the immortalized B cells, and optionally modifying said genes; expressing said genes or modified forms thereof to produce immunoglobulin or analog; and

recovering said immunoglobulin or analog.

4. The method of claim 1 wherein said recovering step comprises
- 5 recovering genes encoding at least the variable region of immunoglobulins from the primary B cells of the animal;
- generating a library of said genes expressing the variable regions;
- 10 screening the library for a variable region with desired affinity for the antigen;
- recovering the genes encoding said variable regions;
- expressing said recovered genes to produce an immunoglobulin or analog containing said variable region
- 15 and recovering said immunoglobulin or analog.

5. A recombinant DNA molecule comprising a nucleotide sequence encoding the immunoglobulin or analog produced by the method of claim 1.

6. The DNA molecule of claim 5 wherein said
- 20 encoding nucleotide sequence is operably linked to control sequences capable of effecting its expression.

7. A cell or cell line modified to contain the DNA molecule of claim 6.

8. A method to produce an immunoglobulin with
- 25 fully human variable region or an analog thereof which method comprises culturing the cells of claim 7 under

conditions whereby said encoding nucleotide sequence is expressed to produce said immunoglobulin or analog; and recovering said immunoglobulin or analog.

9. A DNA molecule comprising a nucleotide
5 sequence corresponding to the gene or modified gene prepared by the method of claim 3.

10. The DNA molecule of claim 9 wherein said encoding nucleotide sequence is operably linked to control sequences capable of effecting its expression.

10 11. A cell or cell line modified to contain the DNA molecule of claim 9.

12. A method to produce an immunoglobulin with fully human variable regions or an analog thereof which method comprises culturing the cells of claim 10 under
15 conditions whereby said encoding nucleotide sequence is expressed to produce said immunoglobulin or analog; and recovering said immunoglobulin or analog.

13. A DNA molecule which comprises a nucleotide sequence encoding a variable region with desired affinity
20 prepared according to the method of claim 4.

14. The DNA molecule of claim 13 wherein said encoding nucleotide sequence is operably linked to control sequences capable of effecting its expression.

15. A cell or cell line modified to contain the DNA molecule of claim 13.

16. A method to produce an immunoglobulin with fully human variable region or an analog thereof which
5 method comprises culturing the cells of claim 14 under conditions whereby said encoding nucleotide sequence is expressed to produce said immunoglobulin or analog; and recovering said immunoglobulin or analog.

17. An immortalized B cell which secretes an
10 immunoglobulin to a desired antigen with a fully human variable region prepared according to claim 3.

18. A method to produce an immunoglobulin or analog which comprises culturing the cells of claim 17 and recovering said immunoglobulin or analog.

19. An immunoglobulin with fully human variable
15 region or analog produced by the method of claim 1.

20. The immunoglobulin or analog of claim 19 wherein the desired antigen is selected from the group consisting of

20 the leukocyte markers, CD2, CD3, CD4, CD5, CD6, CD7, CD8, CD11a,b,c, CD13, CD14, CD18, CD19, CD20, CD22, CD23, CD27 and its ligand, CD28 and its ligands B7.1, B7.2, B7.3, CD29 and its ligand, CD30 and its ligand, CD40 and its ligand gp39, CD44, CD45 and isoforms, CDw52
25 (Campath antigen), CD56, CD58, CD69, CD72, CTLA-4, LFA-1 and TCR;

the histocompatibility antigens, MHC class I or II, the Lewis Y antigens, SLex, SLey, SLea, and SLeb;
the integrins, VLA-1, VLA-2, VLA-3, VLA-4, VLA-5, VLA-6, and LFA-1;
5 the adhesion molecules, Mac-1 and p150,95;
the selectins, L-selectin, P-selectin, and E-selectin and their counterreceptors VCAM-1, ICAM-1, ICAM-2, and LFA-3;
the interleukins, IL-1, IL-2, IL-3, IL-4, IL-5,
10 IL-6, IL-7, IL-8, IL-10, IL-11, IL-12, IL-13, IL-14, and IL-15;
interleukin receptors, IL-1R, IL-2R, IL-4R, IL-5R, IL-6R, IL-7R, IL-8R, IL-10R, IL-11R, IL-12R, IL-13R, IL-14R, and IL-15R;
15 chemokines, PF4, RANTES, MIP1 α , MCP1, NAP-2, Gro α , Gro β , and IL-8;
growth factors, TNF α , TGF β , TSH, VEGF/VPF, PTHrP, EGF family, FGF, PDGF family, endothelin, and gastrin releasing peptide (GRP);
20 growth factor receptors, TNF α R, RGF β R, TSHR, VEGFR/VPFR, FGFR, EGFR, PTHrPR, PDGFR family, EPO-R, GCSF-R and other hematopoietic receptors;
interferon receptors, IFN α R, IFN β R, and IFN γ R;
Igs and their receptors, IgE, Fc ϵ RI, and Fc ϵ RII;
25 tumor antigens, her2-neu, mucin, CEA and endosialin;
the allergens, house dust mite antigen, lol p1 (grass) antigens, and urushiol;
the viral proteins, CMV glycoproteins B, H, and
30 gCIII, HIV-1 envelope glycoproteins, RSV envelope glycoproteins, HSV envelope glycoproteins, EBV envelope

glycoproteins, VZV envelope glycoproteins, HPV envelope glycoproteins, Hepatitis family surface antigens;

the toxins, pseudomonas endotoxin and osteopontin/uropontin, snake venom, and bee venom;

5 the blood factors, complement C3b, complement C5a, complement C5b-9, Rh factor, fibrinogen, fibrin, and myelin associated growth inhibitor;

the enzymes, cholesterol ester transfer protein, membrane bound matrix metalloproteases, and Glutamic acid
10 decarboxylase (GAD); and

the miscellaneous antigens ganglioside GD3, ganglioside GM2, LMP1, LMP2, eosinophil major basic protein, eosinophil cationic protein, pANCA, Amadori protein, Type IV collagen, glycated lipids, γ -interferon,
15 A7, P-glycoprotein and Fas (AFO-1) and oxidized-LDL.

21. A DNA molecule comprising a nucleotide sequence that encodes the immunoglobulin or analog of claim 19 or 20.

22. The DNA molecule of claim 21 wherein said
20 encoding nucleotide sequence is operably linked to control sequences capable of effecting its expression.

23. A cell or cell line modified to contain the DNA molecule of claim 22.

24. A method to produce an immunoglobulin or
25 analog specific for the antigen selected from the group consisting of leukocyte markers, CD2, CD3, CD4, CD5, CD6, CD7, CD8, CD11a,b,c, CD13, CD14, CD18, CD19, CD20, CD22,

CD23, CD27 and its ligand, CD28 and its ligands B7.1,
B7.2, B7.3, CD29 and its ligand, CD30 and its ligand, CD40
and its ligand gp39, CD44, CD45 and isoforms, CDw52
(Campath antigen), CD56, CD58, CD69, CD72, CTLA-4, LFA-1
5 and TCR

histocompatibility antigens, MHC class I or II,
the Lewis X antigens, SLex, SLe^y, SLe^a, and SLe^b;
integrins, VLA-1, VLA-2, VLA-3, VLA-4, VLA-5,
VLA-6, and LFA-1;
10 adhesion molecules, Mac-1 and p150,95;
selectins, L-selectin, P-selectin, and
E-selectin and their counterreceptors VCAM-1, ICAM-1,
ICAM-2, and LFA-3;
interleukins, IL-1, IL-2, IL-3, IL-4, IL-5,
15 IL-6, IL-7, IL-8, IL-10, IL-11, IL-12, IL-13, IL-14, and
IL-15;
interleukin receptors, IL-1R, IL-2R, IL-4R,
IL-5R, IL-6R, IL-7R, IL-8R, IL-10R, IL-11R, IL-12R,
IL-13R, IL-14R, and IL-15R;
20 chemokines, PF4, RANTES, MIP1 α , MCP1, NAP-2,
Gro α , Gro β , and IL-8;
growth factors, TNF α , TGF β , TSH,
VEGF/VPF, PTHrP, EGF family, FGF, PDGF family, endothelin,
and gastrin releasing peptide (GRP);
25 growth factor receptors, TNF α R, RGF β R,
TSHR, VEGFR/VPFR, FGFR, EGFR, PTHrPR, PDGFR family, EPO-R,
GCSF-R and other hematopoietic receptors;
integrin receptors, IFN α R, IFN β R, and IFN γ R;
Igs and their receptors, IgE, Fc ϵ RI, and Fc ϵ RII;
30 tumor antigens, her2-neu, mucin, CEA and
endosialin;

allergens, house dust mite antigen, lol p1
(grass) antigens, and urushiol;

viral proteins, CMV glycoproteins B, H, and
gCIII, HIV-1 envelope glycoproteins, RSV envelope
5 glycoproteins, HSV envelope glycoproteins, EBV envelope
glycoproteins, VZV envelope glycoproteins, HPV envelope
glycoproteins, Hepatitis family surface antigens;

toxins, pseudomonas endotoxin and
osteopontin/uropontin, snake venom, and bee venom;
10 blood factors, complement C3b, complement C5a,
complement C5b-9, Rh factor, fibrinogen, fibrin, and
myelin associated growth inhibitor;

enzymes, cholesterol ester transfer protein,
membrane bound matrix metalloproteases, and glutamic acid
15 decarboxylase (GAD); and

miscellaneous antigens, ganglioside GD3,
ganglioside GM2, LMP1, LMP2, eosinophil major basic
protein, eosinophil cationic protein, pANCA, Amadori
protein, Type IV collagen, glycated lipids, γ -interferon,
20 A7, P-glycoprotein and Fas (AFO-1) and oxidized-LDL

which method comprises culturing the cell or
cell line of claim 23 under conditions wherein said
nucleotide sequence is expressed to produce said
immunoglobulin or analog; and

25 recovering the immunoglobulin or analog.

25. The immunoglobulin or analog of claim 19
wherein said desired antigen is selected from the group
consisting of human IL-6, human IL-8, human TNF α , human
CD4, human L-selectin, human gp39 and tetanus toxin
30 C(TTC).

26. A antibody containing a fully human variable region or analog thereof which is specifically immunoreactive with an antigen selected from the group consisting of human IL-6, human IL-8, human TNF α , human
5 CD4, human L-selectin, human gp39 and tetanus toxin C(TTC).

27. The antibody or analog of claim 26 wherein the desired antigen is human IL-6.

28. The antibody or analog of claim 26 wherein
10 the desired antigen is human IL-8.

29. The antibody or analog of claim 26 wherein the desired antigen is human TNF α .

30. The antibody or analog of claim 26 wherein the desired antigen is human CD4.

15 31. The antibody or analog of claim 26 wherein the desired antigen is human L-selectin.

32. The antibody or analog of claim 26 wherein the desired antigen is human gp39.

33. The antibody or analog of claim 26 wherein
20 the desired antigen is tetanus toxin C(TTC).

34. The analog of claim 26 which is a single chain F_v.

35. A recombinant DNA molecule encoding the antibody or analog of claim 26.

36. A recombinant DNA molecule which comprises an expression system for the production of the antibody or
5 analog of claim 26 which expression system comprises a nucleotide sequence encoding said antibody or analog operably linked to control sequences capable of effecting its expression.

37. A recombinant host cell which is modified
10 to contain the DNA molecule of claim 36.

38. A method to produce an antibody or analog immunospecific for an antigen selected from the group consisting of human IL-6, human IL-8, human TNF α , human CD4, human L-selectin, human gp39 and tetanus toxin
15 C(TTC), which method comprises culturing the cells of claim 37 under conditions wherein said coding sequence is expressed; and
recovering the antibody or analog produced.

39. Use of the antibody or analog of claim 27,
20 29, 31 or 32 for treating an autoimmune disease in a mammal.

40. The use of claim 39 wherein the autoimmune disease is systemic lupus erythrematosus, rheumatoid arthritis, psoriasis, Sjogren's syndrome, scleroderma,
25 mixed connective tissue disease, dermatomyositis, polymyositis, Reiter's syndrome, Behcet's disease, Type I

diabetes, Hashimoto's thyroiditis, Graves' disease, multiple sclerosis, myasthenia gravis, or pemphigus.

41. Use of the antibody of claim 32 for treating graft versus host disease for preventing
5 rejection of an organ transplant, or for treating glomerular nephritis in a mammal.

42. Use of the antibody of claim 31 for treating reperfusion ischemia in a mammal.

43. Use of the antibody of claim 27 for
10 treating cachexia, septic shock, myeloma, renal cell carcinoma, osteoporosis, or Paget disease in a mammal.

44. Use of the antibody of claim 29 for treating septic shock, cachexia, osteoporosis, or systemic sclerosis in a mammal.

15 45. Use of the antibody of claim 28 for preventing tumor metastasis, treating asthma, rheumatoid arthritis, glomerulonephritis, reperfusion injury, adult respiratory distress syndrome, or systemic sclerosis in a mammal.